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Chloroquine Phosphate: A Long-term Follow-up of Drug Concentrations in Skin Suction Blister Fluid and Plasma

GUNILLA SJÖLIN-FORSBERG[1], BERIT BERNE[1], CATARINA BLIXT[2], MONIKA JOHANSSON[2] and BJÖRN LINDSTRÖM[2]†

[1]*Department of Dermatology, University Hospital and* [2]*National Medical Products Agency, Uppsala, Sweden*

Chloroquine is known to bind strongly to melanin and is accumulated in the skin. In dermatology, the drug is mainly used to treat photosensitivity disorders, but it has also been reported to cause sun sensitivity, especially in patients with rheumatoid arthritis. In the present study the concentrations of chloroquine phosphate in plasma and skin suction blister fluid (interstitial fluid in the skin) from 16 patients were studied by HPLC at steady-state (after 2 months' ingestion of 250 mg of the drug daily) and 2, 4 and 6–7 months after cessation of therapy. At steady-state the concentrations were similar in the two compartments, whereas after discontinuation the drug remained much longer in the skin than in the plasma. In tests using cow's eye melanin *in vitro*, UV irradiation failed to interact with the binding of chloroquine to melanin. It is speculated that the prolonged storage of the drug in the skin could be of importance for its therapeutic as well as adverse effects. ***Key words: Melanin; Photoprotection; Photosensitivity.***

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G. Sjölin-Forsberg, Department of Dermatology, University Hospital, S-751 85 Uppsala, Sweden.

The pharmacokinetics of chloroquine phosphate is characterized by rapid absorption from the gastrointestinal tract, plasma protein binding of 55–60%, a high volume of distribution, high plasma clearance and an extremely long half-life (1, 2). Circulating chloroquine binds to a great extent to thrombocytes and granulocytes, resulting in a much higher concentration of the drug in whole blood than in the serum or plasma (3). The concentration of chloroquine is twice as high in the serum as in the plasma, probably as a result of release of the drug from the thrombocytes during the coagulation process (3).

During administration of daily doses, the plasma concentration increases to a steady state after some weeks, but remains relatively low, while some organs such as the kidney, liver, lung and spleen store chloroquine in large amounts (4). The drug is also known to bind strongly to melanin (5) and to accumulate in tissues with a high melanin content such as the eye and the skin (6, 7).

In dermatology, chloroquine is mainly used to decrease photosensitivity in patients with polymorphic light eruption (8), porphyria cutanea tarda (9) and lupus erythematosus (10). On the other hand, the drug has long been suspected of causing photosensitivity in patients with rheumatoid arthritis (11).

In view of the strong melanin-binding capacity of chloroquine and with regard to the controversial issue of its relation to ultraviolet radiation, it seemed of interest to compare the chloroquine concentrations in the circulation (plasma) and in the skin (suction blister fluid) at steady-state and during the phase of elimination and to determine whether UV irradiation could interact with the binding of the drug to melanin.

MATERIAL AND METHODS

In vivo experiments

Sixteen patients, 1 man and 15 women (mean age 42, range 19–56 years), participated in the study, which was approved by the local ethics committee. Six of the patients had a diagnosis of discoid lupus erythematosus, 4 polymorphic light eruption, 3 rheumatoid arthritis, 2 systemic lupus erythematosus and 1 patient subacute cutaneous lupus erythematosus. Two of the patients were having concomitant treatment with non-steroidal antiinflammatory drugs on both light-testing occasions and one patient had a daily dose of 5 mg of prednisolone during the study. All 16 patients were on the point of starting treatment with chloroquine phosphate in a daily dose of 250 mg.

In 7 patients samples of plasma, serum and suction blister fluid were collected before treatment and during steady-state (after 2 months of treatment). Five of these 7 and another 9 patients treated for at least 2 months were divided into three groups and corresponding samples were taken 2 ($n=3$), 4–4.5 ($n=5$) and 6–7 ($n=6$) months, respectively after termination of the chloroquine treatment.

The samples of suction blister fluid were taken from uninvolved, untanned abdominal skin. The suction blisters were induced at the dermal-epidermal junction by a negative pressure of 200–300 mm Hg, according to the method described by Kiistala (12). The content of a fresh blister represents interstitial fluid from the skin (13), thus a fluid sparse in cells (12, 14).

After a standardized centrifugation (10 min at 2,000 g) (15), the blood samples were immediately stored and kept frozen at −70°C until analysed. Chloroquine was extracted from the different samples and subjected to reversed HPLC as described by Alvan et al. (16).

In vitro experiments

In view of the different responses to ultraviolet irradiation during chloroquine treatment, the binding of the drug to melanin with and without preceding irradiation with UVA and UVB was studied as follows: Chloroquine phosphate was prepared in two aqueous solutions of 0.01 and 0.001 M in racemic form. Melanin was obtained from cow's eye (a generous gift from B. Larsson) and dissolved in an aqueous solution as described by Larsson & Tjälve (5).

An Ultravitalux lamp (Osram, Germany) emitting 0.9 mW/cm^2 at a treatment distance of 30 cm was used as a UVB source, and a UV 800 lamp (Waldmann, Germany) emitting 7 mW/cm^2 at a treatment distance of 20 cm served as a source of UVA.

The binding of chloroquine phosphate to melanin was studied as follows: To 0.5 ml of each of a 0.01 M and a 0.001 M solution of chloroquine phosphate, 0.5 ml of a melanin granule suspension (10 ng/ml) was added. Aliquots (100 µl) were pipetted into each of nine open Petri dishes and covered with a clear plastic film (Glad Pack, Union Carbide, Dusseldorf, Germany) which does not absorb ultraviolet irradiation (not shown). The first group of samples ($n=3$) was irradiated with 0.54 J/cm^2 UVB and the second group ($n=3$) with 8.4 J/cm^2 UVA. During irradiation the solutions were continuously stirred. The third group of samples ($n=3$) served as nonirradiated controls and were only stirred. All the samples were then extracted by diethylether (5 ml) after the addition of 0.5 ml NaCl – 0.05 M carbonic buffer (pH 11). After

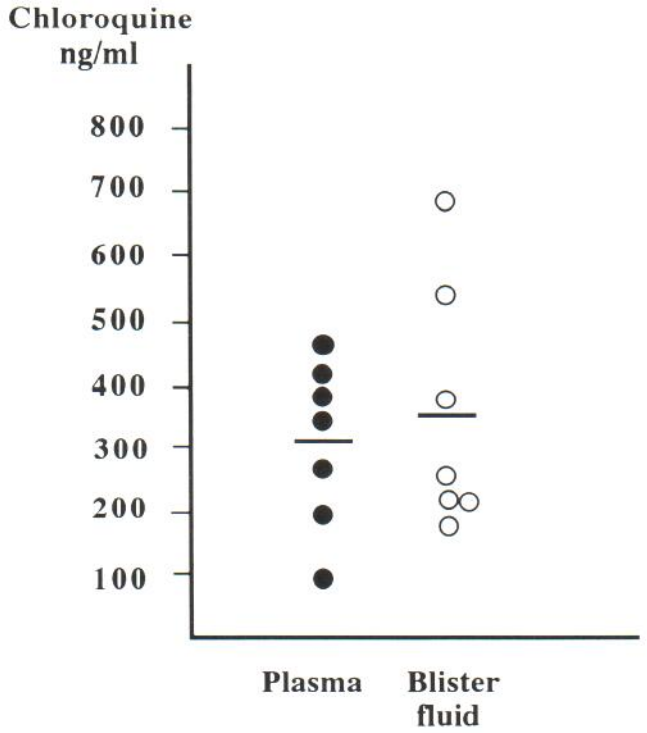

Fig. 1. Concentrations of chloroquine in plasma (●) and skin suction blister fluid (○) during steady-state (after 2 months of 250 mg of chloroquine phosphate daily). Horizontal bars indicate mean values.

centrifugation the ether layer was removed and evaporated. The residue was dissolved in 5 ml of eluent consisting of acetonitrile, methanol and diethylamine (80:19.5:0.5%). Samples of 20 µl, representing the free unbound amount of chloroquine, were injected onto the HPLC column as described earlier (16).

Statistical analysis

In the *in vivo* study a non-parametric method (Wilcoxon rank sum test) was used and a corresponding 95% confidence interval was calculated. In the *in vitro* experiments Student's *t*-test with a 95% confidence interval was used.

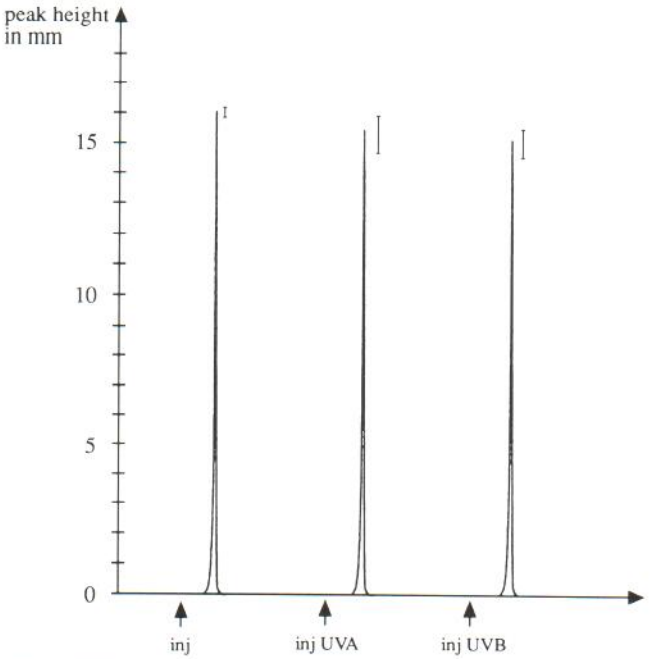

Fig. 3. HPLC chromatogram showing the mean peak heights ($n = 3$) of extracted chloroquine before and after irradiation of the chloroquine-melanin suspension with UVA and UVB. Vertical bars indicate ranges.

RESULTS

In vivo experiments

In accordance with previous reports (3), the concentration of chloroquine at steady-state was higher in serum than in plasma (not shown).

Plasma was chosen as the compartment for comparison with suction blister fluid since both fluids have a sparsity of cells and the interaction of thrombocytes during coagulation was to be avoided. As shown in Fig. 1, at steady-state the chloroquine concentrations in the plasma did not differ significantly from

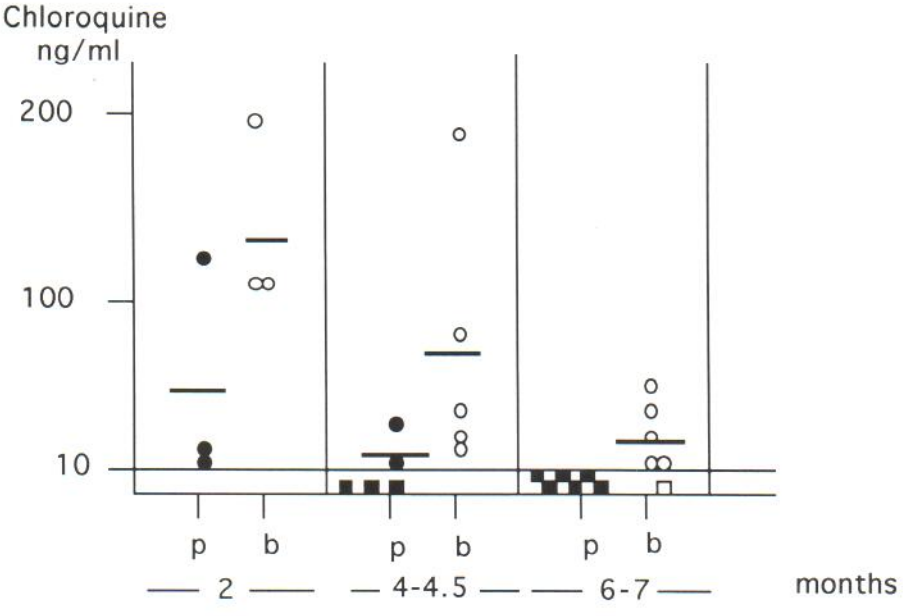

Fig. 2. Concentrations of chloroquine in plasma (*p*) and skin suction blister fluid (*b*) at 2 ($n = 3$), 4–4.5 ($n = 5$) and 6–7 months ($n = 6$) after cessation of chloroquine treatment. Plasma ●, blister fluid ○. Values below the limit of quantitation (10 ng/ml): plasma ■, blister fluid □. Horizontal bars indicate mean values.

that in the suction blister fluid (confidence interval nonparametric method 0.5; 1.73. (95.2%)).

The concentrations of chloroquine in the plasma and suction blister fluid 2, 4 and 6–7 months after cessation of treatment are compared in Fig. 2. The differences are statistically significant (confidence interval, nonparametric method, 0.21; 0.55 (95.3%)). After 4 months a low concentration of chloroquine was found in the plasma of only 2/5 patients, while it was detected in the blister fluid from all the 5 patients. After 7 months there were no detectable concentrations in the plasma but in 5/6 of the blister fluid samples the plasma concentrations were detectable.

The results did not differ between the different categories of patients either at steady-state or after cessation of chloroquine treatment, but with the small numbers of patients (1–4) in these categories this could hardly be expected.

In vitro experiments

Regarding the melanin-binding capacity, no difference in the amount of extracted chloroquine phosphate was found between irradiated and nonirradiated samples as shown in the chromatogram in Fig. 3 (95% confidence interval).

DISCUSSION

From a dermatological point of view a most interesting property of chloroquine is its strong capacity for binding to melanin, with resulting accumulation in the skin. We are not aware of any recent studies on the distribution of chloroquine in the skin. In reports from the fifties and the sixties on the accumulation of the drug in tissues (17, 18), the techniques used had a lower sensitivity and selectivity than the more newly developed ones.

In the present study the concentration of chloroquine in skin suction blister fluid, representing the extracellular fluid just beneath the epidermis, has been analysed by the sensitive and selective technique of HPLC. Furthermore, a direct comparison of the chloroquine concentrations in the skin and plasma at steady-state and at different intervals after cessation of therapy has been made for the first time. During steady-state the concentrations of the drug were similar in the plasma and suction blister fluid (Fig. 1), but after discontinuation the drug was found to be eliminated much more rapidly from the circulation than from the blister fluid, where it could be detected up to 7 months after the treatment had been stopped (Fig. 2). A plausible explanation for these findings could be the large volume of distribution and a slow release of the drug into the interstitial fluids within the skin where the drug is being stored.

The fact that chloroquine is still present in a high concentration in the skin even many months after withdrawal indicates that the drug can exert its effects and side effects even after the treatment has been stopped. This accumulation of chloroquine in the skin is interesting in consideration of its sometimes contradictory effects on the skin in different disorders. The drug can be successfully used for the treatment of photosensitivity in polymorphic light eruption, porphyria cutanea tarda and lupus erythematosus, but its mechanism of action is not known. It would be tempting to ascribe its effect to the accumulation in the skin, but although chloroquine is incorporated into the epidermis (17), where a considerable absorption of ultraviolet light takes place, it does not seem to alter either the erythema-producing ability of ultraviolet irradiation in normal subjects (19) or the absorption characteristics of isolated human epidermis (17). It is also considered unlikely that a mere filtering effect may explain the effects of the drug in these conditions (17). On the other hand, chloroquine has been shown to inhibit certain biochemical changes in the epidermis that follow ultraviolet exposure (20).

The question of whether chloroquine can cause photosensitivity in patients with rheumatoid arthritis has been debated, and in a recent study (21) assessments of the minimal erythema thresholds and provocation with high dose UVA and UVB were found to show similar results with and without chloroquine treatment. Interestingly, a greater number of these patients than expected gave a history of polymorphous light eruption.

As the strong melanin binding is likely to affect the long-standing concentration of chloroquine in the skin, and as long-term administration of drugs with a high melanin affinity may induce toxic lesions in the melanin-containing tissues (5), we were interested in determining whether UV irradiation could affect this binding. Using cow's eye melanin in vitro, no such effects were found, but studies on human melanocytes are in progress.

It is thus difficult to explain how chloroquine can both protect from and induce photosensitivity, but it may be speculated that the prolonged storage of the drug in the skin could be of importance both for the positive and negative effects.

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